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Highlights of the Keystone Symposium: sirtuins in metabolism, aging and disease

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From February 12–16, 2012, leading members of the sirtuin scientific community assembled in Tahoe, CA to attend the Keystone Symposium "Sirtuins in Aging, Metabolism, and Disease." It was a vibrant and lively meeting, and in the spirit of Keystone Symposia, both established sirtuin researchers and those new to the field enjoyed a unique opportunity to interact and exchange ideas.

Aleksey Kazantsev opened the Symposium by highlighting the explosion of research on sirtuins, the evolutionarily conserved NAD^+(nicotinamide adenine dinucleotide)-dependent protein deacetylases that are homologues of the yeast longevity gene Sir2. In the Keynote Address, **Leonard Guarente** provided an overview of the sirtuin deacetylases, which act on a wide variety of protein targets including histones, transcription factors and apoptotic modulators. Moreover, some sirtuins possess other enzymatic activities, such as the mono-ADP-ribosyl transferase activity attributed to mammalian SIRT4. Dr. Guarente described the sirtuins as key sensors for available energy stores that function as a link between protein acetylation and metabolism. Referencing the ongoing debate over the role of sirtuins in the positive responses to calorie restriction (CR), he emphasized that most available data in yeast and invertebrates strongly suggest that the beneficial effects of CR require sirtuin activity and stressed the importance of extending these findings to mammals. Dr. Guarente also discussed evidence from current studies demonstrating that (i) sirtuins appear to suppress diseases of aging; (ii) sirtuin activity declines with aging; and (iii) sirtuins are novel therapeutic targets for many age-related diseases. More specifically, he showed compelling evidence that mammalian SIRT1 activity protects against age-associated pathologies such as diabetes and obesity.

Sirtuins in metabolism

The emerging roles of sirtuins, particularly mammalian SIRT1, in regulating both cellular and organismal metabolism were emphasized throughout the meeting. **John Denu** discussed the role of SIRT1 as a major regulator of central cellular metabolic pathways, focusing on new protein targets of this enzyme. He showed that SIRT1 deacetylates and thereby downregulates activity of phosphoglycerate mutase 1 (PGAM1), an enzyme not thought to be regulated in the glycolytic pathway but now known to catalyse a rate-limiting step of glycolysis in cancer cells. This raises the possibility that reduced activity of SIRT1 in high-glucose conditions results in hyperacetylated PGAM1, which increases energy production via the glycolytic pathway.

Intriguing insights have been gleaned from new mouse models – developed to circumvent the embryonic lethality of SIRT1 knockout (KO) mice – that either lack or overexpress SIRT1 in a tissue-specific and/or inducible manner. **Vittorio Sartorelli** described a tamoxifen-inducible muscle-specific SIRT1 KO mouse. On a high-fat diet (HFD), these KO mice gain more weight than their wild-type (WT) counterparts and exhibit decreased peripheral insulin sensitivity and increased fatty acid (FA) accumulation. KO mice also show marked reduction in the expression of enzymes involved in oxidative phosphorylation. This study suggests that muscle SIRT1 is important for adaptation to HFD and that its loss is sufficient to induce a pathologic response.

David Sinclair described studies in tamoxifen-inducible whole body SIRT1 KO mice: WT and KO mice were fed either a standard diet (SD) or a HFD, with or without supplementation with the natural polyphenol compound resveratrol. SIRT1 KO mice showed decreased mitochondrial function on SD, similar to WT mice on HFD. Mitochondrial function improved in WT, but not KO, mice in response to low-dose resveratrol treatment, suggesting that these effects are

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SIRT1-dependent; however, treatment with high-dose resveratrol showed SIRT1-independent effects, presumably due to the compound acting on other targets.

Rafael de Cabo further illuminated the role of SIRT1 in regulating metabolism and promoting healthy aging, describing longitudinal studies in mice and primates treated with resveratrol and other SIRT1-activating compounds (STACs). In mice, resveratrol treatment of HFD-fed animals normalized both survival and overall metabolic indicators to those observed in animals fed SD without resveratrol. Dr. de Cabo emphasized that, although resveratrol likely acts via multiple targets to exert broad effects, significant benefits of this compound have been observed in many models. Further, the novel STAC SRT1720 also exerts positive effects in HFD-challenged mice: treated animals exhibited a slight decrease in body weight, improved insulin sensitivity, and decreased incidence of cataracts.

Sirtuins in cardiovascular disease

Other speakers highlighted the role of SIRT1 and microRNAs in age-dependent cardiovascular disease (CVD). **Stefanie Dimmeler** pointed out that age is the biggest risk factor for CVD in humans and is associated with increased cardiac fibrosis, apoptosis, heart failure, and decreased neovascularization after ischemia. Several microRNAs have been shown to be regulated by aging; notably, a subset of these microRNAs also control SIRT1 expression and cardiovascular functions. In line with these observations, **Lindsey Gano** showed that age-related vascular endothelial dysfunction, an indicated by impaired endothelium-dependent dilation (EDD), was ameliorated in aged mice treated with the STAC SRT1720. Improvement in EDD was accompanied by restoration of aortic SIRT1 activity in treated animals.

SIRT1-activating compounds

Several presentations directly addressed the ongoing debate over the mechanism(s) of action of resveratrol and other STACs. **David Sinclair** and **George Vlasuk** both showed data demonstrating that a specific amino acid residue, E230, which lies in a structured amino-terminal region of SIRT1, is required for activation via resveratrol and other STACs developed by Sirtris/GSK. These results suggest that STACs that fail to activate E230 mutants are direct activators of SIRT1.

However, **Jay Chung** questioned whether STACs such as resveratrol directly activate SIRT1 *in vivo*, or whether these compounds in fact act on upstream molecules that subsequently activate SIRT1. In Dr. Chung's studies, both resveratrol and SRT1720 were robust AMPK activators and inhibited cAMP phosphodiesterases (PDEs). He suggested that the predominant effect of resveratrol is PDE inhibition and downstream AMPK activation. Others disagreed with that interpretation, arguing that some STAC compounds lack PDE binding. Moreover, **Clemens Steegborn** showed data indicating that modulation of SIRT1 by resveratrol is substrate-specific and that this effect might be due to compound interaction with both the enzyme and its substrate. There was general agreement among participants that the extent to which STACs act directly on sirtuins will be critical to the development of these molecules for therapeutic application in human patients.

Mitochondrial and other sirtuins

Several talks focused on the roles of the mitochondrial sirtuins, particularly SIRT3, in regulating cellular metabolism. **Eric Verdin** showed that mitochondrial proteins from SIRT3 KO mice are hyperacetylated, suggesting that SIRT3 is the major mitochondrial protein deacetylase. SIRT3 is induced during fasting and downregulated in animals fed HFD. The Verdin lab has generated new Cre-dependent SIRT3-overexpressing transgenic mouse lines that will be used to determine whether overexpression of SIRT3 increases insulin sensitivity and FA oxidation and decreases reactive oxygen species (ROS) production. Further, a proteomics approach identified many proteins involved in FA oxidation and amino acid metabolism as potential SIRT3 targets. In accordance with these results, **Marcia Haigis** reported metabolomics studies showing that loss of SIRT3 upregulates glycolysis via stabilization of HIF-1α. Dr. Haigis suggested that stabilization of HIF-1α even in high oxygen conditions could account in part for the ''Warburg Effect'' observed in cancer cells. Finally, **Andrew Dillin** provided compelling evidence that cells in multicellular organisms might orchestrate their responses to energetic challenge via humoral control of mitochondrial form and function.

New insights were gained regarding the activities of some of the less well-understood sirtuins. **Eric Verdin** described a novel activity for SIRT5 as a protein demalonylase as well as a desuccinylase. **Hening Lin** also showed that SIRT5 is an efficient desuccinylase/demalonylase and that other sirtuins that are weak deacetylases have novel activities as well.

Sirtuins in metabolism and cancer

Several speakers described emerging findings linking the chromatin-associated sirtuin SIRT6 to metabolism and cancer. **John Dominy** showed that PGC-1α, a well-known deacetylation target of SIRT1, is hyperacetylated upon overexpression of SIRT6. **Haim Cohen** described results from SIRT6-overexpressing mice, which were previously shown to be resistant to the negative effects of HFD. Increased SIRT6 expression extends lifespan in male but not female mice; as a result, SIRT6-overexpressing males now live as long as females. Extension of lifespan in males on HFD is associated with improved glucose homeostasis and decreased serum IGF1, such that levels in males become comparable to that observed in females. Dr. Cohen pointed out that this ''feminization'' of gene expression patterns is also observed in males after CR.

Katrin Chua and **Raul Mostoslasky** both described findings in SIRT6 KO mice, which show genomic instability

and increased hypersensitivity to DNA damage as well as acute degenerative and metabolic pathology; these animals die at 4 weeks of age due to hypoglycemic crash. Dr. Chua reported that the early lethality of SIRT6 KO mice can be overcome by a temporary dietary regimen rich in fat and glucose; this approach revealed later-life phenotypes of SIRT6-deficiency, including some intriguing behavioural abnormalities. Dr. Chua described her findings that SIRT6 is a histone H3K9 and H3K56 deacetylase that regulates telomeric chromatin and NF-κB-dependent gene expression and then presented new evidence that SIRT6 is important for mitotic fidelity. SIRT6 deficiency leads to mitotic errors and aneuploidy, and SIRT6 KO MEFs show accelerated spontaneous immortalization, a hallmark of cancer cells. Dr. Mostoslavsky showed an increase in glucose uptake in brown adipose tissue (BAT) and muscle in SIRT6 KO mice, and suggested that this increased glucose uptake is responsible for the acute hypoglycemia. SIRT6 KO mice also exhibit increased lactate production and inhibition of mitochondrial respiration, resembling the Warburg Effect in tumour cells. ChIP experiments revealed association of SIRT6 with key genes involved in glycolysis, and SIRT6 effects are mediated by deacetylation of H3K9, which is hyperacetylated in the SIRT6 KO mouse and is associated with increased binding of HIF-1a and activation of glycolytic gene expression. Whether SIRT6 may act as a tumour suppressor by modulating the Warburg Effect remains to be established.

In another link between sirtuins, metabolism and cancer, SIRT3 KO mice develop tumours at 1 year of age. **Marcia Haigis** showed that analysis of human tumours showed SIRT3 LOH in 40% of breast and ovarian tumours and reduced SIRT3 expression (<50%) in 85% of patients. Finally, **Katrin Chua** described a novel activity for SIRT7 in tumourigenesis, showing that this sirtuin selectively deacetylates H3K18 to stabilize the transformed state of cancer cells.

In contrast to the proposed anti-tumourigenic roles proposed for SIRT3, SIRT6, and SIRT7, **Lucia Altucci** and **Alejandro Vaquero** both discussed the therapeutic potential of specific sirtuin inhibitors as anti-cancer agents. Dr. Altucci showed that a dual SIRT1/SIRT2 inhibitor increased caspase activation and cell death in cultured leukaemia cells and induced apoptosis in both tumour cell lines and in cells from primary tumours. The SIRT1/2 inhibitor also exerted anti-tumourigenic effects in allograft and xenograft models *in vivo*. Dr. Vaquero showed that cells from SIRT2 KO mice exhibit increased proliferation, DNA damage, and genomic instability. These changes were associated with hyperacetylation of H4K16. Intriguingly **Mario Fraga** observed decreased SIRT1 RNA and protein levels during human ES cell differentiation and a concomitant increase in acetylation of the putative SIRT1 target H4K16.

Sirtuin modulators

There was a strong focus throughout the meeting on discovery of sirtuin modulators, underscoring the widespread interest in developing sirtuin-based drugs. **Clemens Steegborn** used acetylome peptide microarrays to identify distinct substrate specificities for mammalian sirtuins, suggesting limited direct compensation of sirtuin paralogs. He also described differential inhibition of mammalian sirtuins by nicotinamide, a deacetylation by-product, and different potencies against Sirt5-dependent turnover of acetylated or succinylated substrates. **Antti Poso** used virtual docking approaches to model sirtuin–substrate interactions and described structural features of this interaction that complicate development of novel sirtuin modulators. **Manfred Jung** and **Antonello Mai** both described targeted approaches for developing sirtuin inhibitors. Because kinase inhibitors are ATP mimics that might also interact with NAD+-binding sites, Dr. Jung screened a focused library of kinase inhibitors to identify sirtuin inhibitors and identified a PKC inhibitor with an IC_{50} value of 800 nM for SIRT2. Dr. Mai detailed a medicinal chemistry approach for developing new classes of sirtuin modulators and evaluating their antiproliferative activities. The best effects were achieved with a dual SIRT1/SIRT2 inhibitor, and both activities appear to be required for antiproliferative activity. Dr. Mai noted that some 1,4-dihydropyridine compounds with sirtuin activating properties also show antiproliferative effects. **Yana Cen** described the use of kinetic isotope effects (KIEs) to study biochemical transition states of sirtuins from *Plasmodium* and *Archaeoglobus*, which might aid in designing novel sirtuin inhibitors.

Sirtuins in metabolism, aging and neuronal function

Finally, several presentations suggested a key role for sirtuins in linking metabolism, aging and neuronal function. Describing the 'NAD World' as a systemic regulatory network connecting metabolism and aging, **Shin-ichiro Imai** presented new results from detailed analyses of brain-specific SIRT1-overexpressing (BRASTO) mice, including effects on longevity and age-associated physiological changes. Dr. Imai also suggested a molecular mechanism by which SIRT1 regulates hypothalamic neural activities that affect the aging process and possibly longevity. This presentation stimulated discussions on the importance of sirtuins for the control of aging and longevity in mammals.

Sergiy Libert described two new mouse models in which SIRT1 is either specifically deleted (BSKO) or overexpressed (OX) in brain. BSKO mice exhibit increased exploratory behaviour, while OX mice explore less. Behaviour in OX mice is normalized by SSRI antidepressants, suggesting that OX mice have impaired serotonin signalling. In the forced swim test, BKSO mice are less passive and OX mice are more passive; BSKO mice also have increased levels of serotonin, noradrenalin and 5HAA, and all of these are reduced in OX animals.

Several speakers described efforts to probe the role of sirtuins in neurons and to investigate the therapeutic potential of sirtuin modulators for treating neurological diseases, especially age-dependent neurodegenerative disorders. **Larry Marsh** used a Drosophila model of Huntington's disease (HD) to screen for modifiers of polyglutamine-induced neu-

rotoxicity and identified sir2 (Drosophila homolog of SIRT1) and sirt2. The observed beneficial effects were strikingly dependent on gene dosage: heterozygotes showed improved phenotype but homozygous deletion increased neurodegeneration. Treatment with selistat, a specific sir2 inhibitor, provided dose-dependent neuroprotection but this effect again required some sir2 to be present. Resveratrol was also protective in the fly model but its effects do not appear to require sir2, since they were also observed in sir2−/− animals. In contrast to the beneficial effects of inhibiting sir2 in the fly, **Christian Neri** showed that in *Caenorhabditis elegans*, overexpression of sir2.1 (worm SIRT1) protects against polyglutamine-induced neuronal dysfunction while both sir2.1 loss-of-function and sir-2.1 RNAi exacerbate the phenotype. In a mouse model of HD, **Wenzhen Duan** showed that SIRT1 overexpression is neuroprotective and ameliorates behavioural deficits, perhaps by increasing expression of important neurotrophins such as BDNF. Lastly, **Ruth Luthi-Carter** described a potential mechanism for the benefits of SIRT2 inhibition in a primary striatal neuron model of Huntington's disease. Together, these studies highlighted key questions surrounding efforts to develop sirtuin modulators for treatment of neurological diseases, including whether it is activation or inhibition of specific sirtuins that is beneficial.

Concluding remarks

In his closing remarks, **Rafael de Cabo** emphasized the importance of studying healthy aging, *i.e.* healthspan, in addition to lifespan, arguing that aging is the major risk factor for all human disease and that the goal of aging research is to achieve not more years, but more healthy years. Overall, the results presented at this meeting support the development of sirtuin-based therapies as a promising therapeutic approach for treatment of age-related human diseases.

The authors declare that they have no conflict of interest.